

What Is Claimed:

1. A method of interfering with CD9 binding to fibronectin comprising either (i) contacting a CD9 protein or polypeptide with an agent that binds
5 to a fibronectin-binding domain of the CD9 protein or polypeptide, (ii) contacting fibronectin with a polypeptide fragment of CD9 that includes at least a part of a fibronectin-binding domain, or (iii) both said contacting the CD9 protein or polypeptide and said contacting fibronectin,
wherein each said contacting interferes with CD9 binding to
10 fibronectin.
2. The method according to claim 1 wherein said method is carried out by contacting the CD9 protein or polypeptide with an agent that binds to a fibronectin-binding domain of the CD9 protein or polypeptide.
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3. The method according to claim 2 wherein the agent is an antibody or fragment thereof that binds the amino acid sequence of PKKDV (SEQ ID NO: 3).
- 20 4. The method according to claim 2 wherein the antibody or fragment thereof is an anti-CD9 mAb7 antibody.
5. The method according to claim 1 wherein said method is carried out by contacting fibronectin with a polypeptide fragment of CD9 that
25 comprises at least a part of a fibronectin-binding domain.
6. The method according to claim 5 wherein the polypeptide fragment of CD9 comprises PKKDV (SEQ ID NO: 3).
- 30 7. The method according to claim 5 wherein the polypeptide fragment of CD9 is
KDEPQRETLKAIHYALNCCGLAGGVEQFISDICPKKDV (SEQ ID NO: 4);
PKKDVLETFTVKSCPDAIKEVFDNK (SEQ ID NO: 5);
PKKDVLETFTVKSCPDAI (SEQ ID NO: 6); or
35 a combination thereof.

8. The method according to claim 1 wherein said method is carried out by both said contacting the CD9 protein or polypeptide and said contacting fibronectin.

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9. A method of modifying adhesion, motility, or spreading of a CD9-expressing cell on fibronectin comprising modifying CD9 expression levels or CD9 activity on a CD9-expressing cell, wherein enhanced CD9 expression levels inhibit adhesion of the CD9-expressing cell and enhance motility and spreading of the CD9-expressing cell, and inhibited CD9 activity enhances adhesion of the CD9-expressing cell and inhibits motility and spreading of the CD9-expressing cell.

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10. The method according to claim 9 wherein said modifying CD9 expression levels comprises transforming the CD9-expressing cell with an expression vector encoding CD9 under conditions effective to enhance CD9 expression levels or an expression vector encoding antisense CD9 RNA or siRNA under conditions effective to decrease CD9 expression levels.

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11. The method according to claim 9 wherein said modifying CD9 activity comprises either (i) contacting CD9 with an agent that binds to the CD9 EC2 domain, (ii) contacting fibronectin with one or more polypeptide fragments of CD9 that include at least a part of a fibronectin-binding domain, (iii) contacting an integrin colocalized with CD9 on the cell surface with an agent that binds to the integrin, or (iv) any combination of (i), (ii), and (iii).

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12. The method according to claim 11 wherein said modifying is carried out by contacting CD9 EC2 domains on a cell with an agent that binds to the CD9 EC2 domain.

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13. The method according to claim 12 wherein the agent is an antibody or fragment thereof that binds to the amino acid sequence of PKKDV (SEQ ID NO: 3).

14. The method according to claim 13 wherein the antibody or fragment thereof is an anti-CD9 mAb7 antibody.

15. The method according to claim 11 wherein said modifying is carried out by contacting fibronectin with one or more polypeptide fragments of CD9 that comprises at least a part of a fibronectin-binding domain.

16. The method according to claim 15 wherein the one or more polypeptide fragments of CD9 comprise PKKDV (SEQ ID NO: 3).

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17. The method according to claim 15 wherein the one or more polypeptide fragments of CD9 comprise

KDEPQRETLKAIHYALNCCGLAGGVEQFISDICPKKDV (SEQ ID NO: 4);

PKKDVLETFTVKSCPDAIKEVFDNK (SEQ ID NO: 5);

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PKKDVLETFTVKSCPDAI (SEQ ID NO: 6); and combinations thereof.

18. The method according to claim 11 wherein said modifying is carried out by contacting an integrin colocalized with CD9 on the cell surface with an agent that binds to the integrin.

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19. The method according to claim 18 wherein the integrin is $\alpha 5\beta 1$ integrin.

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20. The method according to claim 19 wherein the agent is polyclonal antibody PB1.

21. The method according to claim 11 wherein said modifying is carried out by any combination of said contacting the CD9 protein or polypeptide, said contacting fibronectin, and said contacting an integrin colocalized with CD9.

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22. The method according to claim 9 wherein the cell is *in vitro*.

23. The method according to claim 9 wherein the cell is *in vivo*.

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24. The method according to claim 9 wherein the cell is a leukocyte, endothelial cell, vascular smooth muscle cell, or glial cell.

25. A method of inhibiting proliferation or survival of CD9-expressing cells, said method comprising either (i) contacting a cell expressing CD9 with an agent that binds to a CD9 extracellular domain, or (ii) contacting a cell expressing CD9 with an inhibitor of PI 3-kinase under conditions effective to cause uptake of the inhibitor, of (iii) both (i) and (ii), wherein each said contacting inhibits proliferation or survival of the cells expressing CD9.

26. The method according to claim 24 wherein the inhibitor of PI 3-kinase is LY294002 or wortmannin.

27. The method according to claim 25 wherein said contacting with an inhibitor of PI3-kinase comprises administering the inhibitor of PI 3-kinase to the subject under conditions effective to allow the inhibitor of PI 3-kinase to contact the cell expressing CD9.

28. The method according to claim 27 wherein said administering is carried out orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intranasal instillation, by intracavitary or intravesical instillation, intraocularly, intraarterially, intralesionally, by application to mucous membranes, such as, that of the nose, throat, and bronchial tubes, or by transdermal delivery.

29. The method according to claim 25 further comprising: contacting an extracellular matrix comprising fibronectin, which extracellular matrix is in contact with the cell expressing CD9, with one or more polypeptide fragments of CD9 that each comprise at least a part of a fibronectin-binding domain.

30. The method according to claim 29 wherein the one or more polypeptide fragments comprise PKKDV (SEQ ID NO: 3).

31. The method according to claim 29 wherein the one or more polypeptide fragments of CD9 is selected from the group consisting of:

KDEPQRETLKAIHYALNCCGLAGGVEQFISDICKPKKDV (SEQ ID NO: 4);

PKKDVLETFTVKSCPDAIKEVFDNK (SEQ ID NO: 5);

PKKDVLETFTVKSCPDAI (SEQ ID NO: 6); and

combinations thereof.

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32. The method according to claim 29 wherein said contacting the extracellular matrix comprises administering the one or more polypeptides to the patient under conditions effective to substantially saturate available CD9 binding sites on the extracellular matrix with the one or more polypeptides.

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33. The method according to claim 32 wherein said administering is carried out orally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intranasal instillation, by intracavitary or intravesical instillation, intraocularly, intraarterially, intralesionally, by application to mucous membranes, such as, that of the nose, throat, and bronchial tubes, or by transdermal delivery.

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34. A method of treating a subject for a condition or disease state involving proliferation or survival of CD9-expressing cells comprising:

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performing the method according to claim 25, wherein inhibiting proliferation or survival of the CD9-expressing cells treats the condition or disease state.

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35. The method according to claim 34 wherein the condition or disease state involving proliferation or survival of CD9-expressing cells is selected from the group consisting of thrombosis, atherosclerosis, vein graft failure, restenosis, transplant arteriopathy, bleeding disorders, angiogenesis, and primary and metastatic cancers.

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36. The method according to claim 35 wherein the condition or disease state involving proliferation of CD9-expressing cells is restenosis and the CD9-expressing cells are vascular smooth muscle cells.

37. The method according to claim 35 wherein the condition or disease state involving proliferation or survival of CD9-expressing cells is primary or

metastatic cancer and the CD9-expressing cells cancer cells are selected from the group consisting of breast cancer, prostate cancer, colon cancer, melanoma, ovarian cancer, neuroblastoma, glioma, and glioblastoma.

5 38. A method of modifying pericellular fibronectin matrix assembly comprising modifying CD9 expression levels or CD9 activity on a CD9-expressing cell, wherein enhanced CD9 expression levels inhibit pericellular matrix assembly and inhibited CD9 activity augments pericellular matrix assembly.

10 39. The method according to claim 38 wherein said modifying comprises transforming the CD9-expressing cell with an expression vector encoding CD9 under conditions effective to enhance CD9 expression levels or with an expression vector encoding CD9 antisense RNA or siRNA under conditions effective to decrease CD9 expression levels.

15 40. The method according to claim 38 wherein said modifying comprises inhibiting CD9 activity.

20 41. The method according to claim 40 wherein said inhibiting comprises either (i) contacting a CD9 protein or polypeptide of a CD9-expressing cell with an agent that binds to a fibronectin-binding domain of the CD9 protein or polypeptide, (ii) contacting the pericellular fibronectin matrix assembly with one or more polypeptide fragments of CD9 that include at least a part of a fibronectin-binding domain, or (iii) both (i) and (ii), wherein each said contacting induces
25 pericellular fibronectin matrix assembly by the CD9-expressing cell.

 42. The method according to claim 41 wherein said inhibiting is carried out by contacting a CD9 protein or polypeptide of a CD9-expressing cell with an agent that binds to a fibronectin-binding domain of the CD9 protein or polypeptide.

30 43. The method according to claim 42 wherein the agent is an antibody or fragment thereof that binds the amino acid sequence of PKKDV (SEQ ID NO: 3).

44. The method according to claim 43 wherein the antibody or fragment thereof is an anti-CD9 mAb7 antibody.

45. The method according to claim 41 wherein said inhibiting is
5 carried out by contacting the pericellular fibronectin matrix assembly with one or more polypeptide fragments of CD9 that include at least a part of a fibronectin-binding domain.

46. The method according to claim 45 wherein the one or more
10 polypeptide fragments of CD9 comprise PKKDV (SEQ ID NO: 3).

47. The method according to claim 45 wherein the one or more polypeptide fragments of CD9 comprise
KDEPQRETLKAIHYALNCCGLAGGVEQFISDICPKKDV (SEQ ID NO: 4);
15 PKKDVLETFTVKSCPDAIKEVFDNK (SEQ ID NO: 5);
PKKDVLETFTVKSCPDAI (SEQ ID NO: 6); or
a combination thereof.

48. The method according to claim 41 wherein said inhibiting is
20 carried out by both said contacting the CD9 protein or polypeptide and said contacting pericellular fibronectin matrix assembly.

49. A method of modifying invasiveness of a cell through a collagen and/or laminin matrix comprising modifying CD9 expression levels or CD9
25 activity on a CD9-expressing cell, wherein enhanced CD9 expression levels inhibit invasiveness and inhibited CD9 activity promotes invasiveness.

50. The method according to claim 49 wherein said modifying comprises transforming the CD9-expressing cell with an expression vector encoding
30 CD9 under conditions effective to enhance CD9 expression levels or with an expression vector encoding CD9 antisense RNA or siRNA under conditions effective to decrease CD9 expression levels.

51. The method according to claim 49 wherein said modifying
35 comprises inhibiting CD9 activity.

52. The method according to claim 51 wherein said inhibiting comprises either (i) contacting a CD9 protein or polypeptide of a CD9-expressing cell with an agent that binds to a fibronectin-binding domain of the CD9 protein or polypeptide, (ii) contacting the pericellular fibronectin matrix assembly with one or more polypeptide fragments of CD9 that include at least a part of a fibronectin-binding domain, or (iii) both (i) and (ii), wherein each said contacting induces pericellular fibronectin matrix assembly by the CD9-expressing cell.

53. The method according to claim 52 wherein said inhibiting is carried out by contacting a CD9 protein or polypeptide of a CD9-expressing cell with an agent that binds to a fibronectin-binding domain of the CD9 protein or polypeptide.

54. The method according to claim 53 wherein the agent is an antibody or fragment thereof that binds the amino acid sequence of PKKDV (SEQ ID NO: 3).

55. The method according to claim 54 wherein the antibody or fragment thereof is an anti-CD9 mAb7 antibody.

56. The method according to claim 52 wherein said inhibiting is carried out by contacting the pericellular fibronectin matrix assembly with one or more polypeptide fragments of CD9 that include at least a part of a fibronectin-binding domain.

57. The method according to claim 56 wherein the one or more polypeptide fragments of CD9 comprise PKKDV (SEQ ID NO: 3).

58. The method according to claim 56 wherein the one or more polypeptide fragments of CD9 comprise

KDEPQRETLKAIHYALNCCGLAGGVEQFISDICKPKDV (SEQ ID NO: 4);

PKKDVLETFTVKSCPDAIKEVFDNK (SEQ ID NO: 5);

PKKDVLETFTVKSCPDAI (SEQ ID NO: 6); or

a combination thereof.

59. The method according to claim 52 wherein said inhibiting is carried out by both said contacting the CD9 protein or polypeptide and said contacting pericellular fibronectin matrix assembly.

5 60. A method of modifying cell-to-cell interaction comprising modifying CD9 expression levels or CD9 activity on a CD9-expressing cell, wherein enhanced CD9 expression levels promote interaction with a second cell possessing a CD9 ligand and inhibited CD9 activity diminishes interaction with the second cell.

10 61. The method according to claim 60 wherein said modifying comprises transforming the CD9-expressing cell with an expression vector encoding CD9 under conditions effective to enhance CD9 expression levels or with an expression vector encoding CD9 antisense RNA or siRNA under conditions effective to decrease CD9 expression levels.

15 62. The method according to claim 60 wherein said modifying comprises inhibiting CD9 activity.

20 63. The method according to claim 62 wherein said inhibiting comprises either (i) contacting a CD9 protein or polypeptide of a CD9-expressing cell with an agent that binds to the CD9 protein or polypeptide, (ii) contacting the target cell with one or more polypeptide fragments of CD9 that include at least a part of an extracellular domain, or (iii) both (i) and (ii).

25 64. The method according to claim 63 wherein said inhibiting is carried out by contacting a CD9 protein or polypeptide of a CD9-expressing cell with an agent that binds to the CD9 protein or polypeptide.

30 65. The method according to claim 64 wherein the agent is an antibody or fragment thereof that binds at least part of EC1 or EC2.

66. The method according to claim 65 wherein the antibody or fragment thereof is an anti-CD9 mAb7 antibody.

67. The method according to claim 63 wherein said inhibiting is carried out by contacting the target cell with one or more polypeptide fragments of CD9 that include at least a part of an extracellular domain.

5 68. The method according to claim 67 wherein the one or more polypeptide fragments of CD9 comprise PKKDV (SEQ ID NO: 3).

69. The method according to claim 67 wherein the one or more polypeptide fragments of CD9 comprise
10 KDEPQRETLKAIHYALNCCGLAGGVEQFISDICPKKDV (SEQ ID NO: 4);
 PKKDVLETFTVKSCPDAIKEVFDNK (SEQ ID NO: 5);
 PKKDVLETFTVKSCPDAI (SEQ ID NO: 6); or
a combination thereof.

15 70. The method according to claim 63 wherein said inhibiting is carried out by both said contacting the CD9 protein or polypeptide and said contacting the target cell with one or more polypeptide fragments of CD9.

71. A method of diagnosing sperm-egg fusion infertility
20 comprising:
 obtaining an egg from a female patient and
 determining the quantity of CD9 expressed on the egg,
 wherein a lower than normal CD9 expression level indicates that the
egg has a reduced opportunity for fusion with a sperm.

25 72. An isolated polypeptide that is a fragment of human CD9 and comprised at least 5 contiguous amino acids from amino acids 35-58 of human CD9 or amino acids 113-192 of human CD9.

30 73. The isolated polypeptide according to claim 72 wherein the polypeptide is

 KDEPQRETLKAIHYALNCCGLAGGVEQFISDICPKKDV (SEQ ID NO: 4);
 PKKDVLETFTVKSCPDAIKEVFDNK (SEQ ID NO: 5); or

PKKDVLETFTVKSCPDAI (SEQ ID NO: 6).

74. The isolated polypeptide according to claim 72 wherein the polypeptide comprises PKKDV (SEQ ID NO: 3).

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75. A chimeric protein comprising the polypeptide according to claim 72.

76. An antibody raised against the polypeptide according to claim 72.

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77. The antibody according to claim 76, wherein the antibody is present in polyclonal form.

78. The antibody according to claim 76 wherein the antibody is present in monoclonal form.

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